A Proposed Approach to Study the Toxicology of Complex Mixtures of Petroleum Products: The Integrated Use of QSAR, Lumping Analysis and PBPK/PD Modeling

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Mixture toxicity is a topic that has become a matter of concern during the last two decades. One of the major problems with assessing the toxicity of mixtures and the associated human and environmental risk is the large number of possible mixtures, as well as the fact that the actual mixture effect for a given set of constituents might strongly depend on the actual composition of the mixture, i.e., the ratios of the constituent, as well as their nature. This paper presents a possible approach to describe and thereby better understand the pharmacokinetics and dynamics of complex mixtures by combining quantitative structure–activity relationships to predict needed parameters, lumping to reduce the complexity of the problem, and physiologically based pharmacokinetic/pharmacodynamic modeling to integrate all this information into a complete toxicological description of the mixture. It is our hope that by presenting this conceptual approach we might be able to stimulate some criticisms and discussions in the toxicology community regarding this complex and yet very important area of research. — Environ Health Perspect 105(Suppl 1):179–195 (1997)

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Introduction

Traditionally, both human and environmental toxicologists have studied the toxic effects (both lethal and sublethal, acute and chronic) of single substances. For decades, the most important toxicological parameter of a compound, and the one that increasingly should at least be determined, was the so-called LD_{50}, or median lethal dose, for mammals and other terrestrial animal species or the LC_{50}, or median lethal aqueous concentration, for aquatic species (including fish, crustaceans, and algae). As the science of toxicology advances, more and more complicated testing methods are developed and utilized, some of which can be extremely animal and resource intensive.

Over the last twenty years or so, however, it has become apparent that conventional toxicologic methodologies are neither practical for nor capable of assessing the toxicity of the huge number of single chemicals, particularly the almost infinite number of chemical mixtures. Thus, protecting both human and environmental health based on conventional toxicity testing may not be achievable. There are two major reasons for this problem.

First, there simply are too many different chemicals to be adequately tested for the large number of recognized or still unknown effects, especially on environmental health, which requires measurements of effect concentrations not only on single species but also on complex biotic communities or ecosystems. For example, the European Inventory of Existing Commercial Chemical Substances (EINECS) contains 100,000 individual entries (these are chemical products that are actually being marketed (*1*); the Chemical Abstracts Registry contains several million substances that have been (tentatively) synthesized in laboratories (*2*); and the Aquatic Toxicity Information Retrieval Database (AQUIRE) created and maintained by the U.S. EPA (*3*), the most comprehensive and reliable source of aquatic toxicity data, contains pertinent experimental data for about 5500 different chemicals.

Second, in real-life situations, humans and the environment normally are not exposed to single toxicants but to complex mixtures of large numbers of potentially hazardous and potentially interacting agents. Some brief examples illustrate this point. First, consider some of the recent thinking about the Persian Gulf War syndrome. It is now hypothesized that the causative agent for some reported illnesses might have been a combination of immunization shots, anti-nerve-gas prophylactics, insecticides, and insect repellents, all of which most Gulf War veterans were expected to apply before active duty (*4,5*). Alternatively, consider the major die-off of harbor seals reported in the Dutch and German section of the Wadden Sea several years ago. The cause of the die-off, which originally stumped marine mammal researchers, finally appeared to be a combination of a normally innocuous mammalian virus infection and the immune system dysregulating effect of a high level of a complex mixture of organochlorine compounds, notably polychlorinated biphenyls (PCBs), followed by opportunistic infections (*6,7*). Last, current understanding of the potential of persistent chlorinated xenobiotics (such as PCBs, dieldrin, and toxaphene) to act as environmental estrogens also includes a strongly synergistic mixture effect (*8*).

It has been recognized for some time that one of the more promising approaches

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Abbreviations: CNS, central nervous system; GC, gas chromatography; GC–MS, gas chromatography–mass spectrometry; IR, infrared spectroscopy; NMR, nuclear magnetic resonance; PAHs, polycyclic aromatic hydrocarbons; PBPK/PD, physiologically based pharmacokinetic/pharmacodynamic modeling; PLS, partial least squares; QSAR, quantitative structure–activity relationship; SAR, structure–activity relationship; SPME, solid phase microextraction equilibration.

to dealing with the first problem, that of the existence of too many compounds to test for toxic effects, is the discipline known as predictive toxicology. This is a discipline that tries to predict the biological effects, including but not limited to acute toxicity, of chemicals from considerations of compound structure and knowledge about the target biological systems and subsystems. Two tools predictive toxicology has developed over the years are quantitative structure-activity relationships (QSAR) and physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling.

QSAR attempts to predict the qualitative structure-activity (SAR) or quantitative QSAR effect of a compound by analogy with a number of similar compounds (*9*). SARs generally define a common substructure or overall shape similarity between compounds with similar modes of toxic action, using the presence or absence of this substructure in an unknown compound or the similarity of the unknown to the known active compounds to predict the likelihood of the unknown of exhibiting the same toxicity. QSARs attempt to identify quantitative structural parameters that correlate with the actual effect dose or concentration that elicits a common effect level (for example, LD_{50} or LC_{50}).

PBPK/PD modeling approaches the issue of predicting biological effects not from the viewpoint of a chemical but from the viewpoint of a biological system. Basically, what a PBPK model does is to describe an organism (or a tissue or a cell) as a connected system of compartments in which mass balances, including transport processes, diffusion exchanges, metabolic and eliminatory clearances, receptor binding, etc., account for the uptake and disposition of chemicals in this organism (*10*). If the PBPK model is verified against experimental data, the pharmacokinetics of the compound can be predicted by the model. This leads to two predictive improvements. First, most of the chemical-specific parameters (blood–tissue and tissue–tissue partition coefficients as well as metabolic and elimination rate constants) for an unknown can be determined *in vitro* and then used to create a predictive model. Second, if the biological specifics of an unknown target organism are available, a model can be adapted to predict the kinetics of a given chemical of interest for this unknown species. Furthermore, if for a specifically acting compound the pharmacodynamics at the target organ or site (e.g., a cell or enzyme) are known, these can be linked to the predicted pharmacokinetics and the complete PBPK/PD model can be employed to predict effect levels for a certain toxic effect. It has even been shown recently that it is possible to incorporate the biological variability found in nonclonal populations of a species into a PBPK/PD model for predictive toxicology by using Monte Carlo simulation techniques (*11,12*).

We describe here an integrated predictive toxicology approach that uses QSAR, PBPK/PD, and lumping analysis techniques to model the toxicologic effects of complex mixtures of chemicals. This approach is based on the concept that PBPK/PD modeling not only can predict the pharmacokinetics and dynamics of single compounds but is in principle also technically capable of incorporating the kinetics of a number of compounds simultaneously. Moreover, if the pharmacodynamics of these compounds are known (from *in vitro* studies or from studies with other species) and their possible interactions with each other have been studied, the overall toxic effect level of any dose or ratio of the mixture can, in principle, be predicted. QSAR techniques are instrumental in predicting the necessary chemical input parameters for unknown compounds such as tissue–blood, tissue–tissue and blood–air (or blood–water) partition coefficients, metabolic rate constants and elimination constants as well as possible pharmacodynamic parameters such as binding affinities and maximum turnover velocities for target enzyme systems. These pharmacodynamic parameters will in most instances automatically yield interactive effects also, either from competitive or noncompetitive binding to targets or from indirect interaction between separate targets.

As mixtures get large (i.e., consisting of more than, say 10 or 20 compounds), however, several problems make even this approach unworkable. For one thing, a general lack of experimental data on individual compounds can make model formulation almost impossible. It becomes even more difficult if one attempts to develop a model for a mixture of unknown, partially known, or undefined and changing composition such as a gasoline or jet fuel mixture. Another limitation in the complexity of the final model and thus in the total number of compounds in a mixture that can adequately be modeled is imposed by the state of the current hardware, and even more importantly, by the state of the current software.* In all these cases the complexity of the model must be reduced, preferably by application of either intrinsic or explicit lumping analysis. Lumping analysis is a technique developed in the petroleum industry to reduce the complexity of a model description of a large mixture to make reaction rate and reaction product profile predictions for technical treatments of mixtures of petroleum products such as the cracking of a certain fraction of a crude (*13*). Lumping can be used to treat compounds with the same or similar partition coefficients as a single surrogate compounds or to treat a set of agonists on a target organ, cell, or enzyme as a single agent with an average affinity or turnover rate.

All three techniques mentioned above will be described in more detail in another section of this paper as will ways in which these techniques might be combined into a comprehensive predictive toxicological approach. These techniques will be illustrated wherever possible by examples using such complex mixtures as JP-5, automotive gasoline, and other petroleum products. Finally, an example will be given of a tentative application of all three techniques to the toxicological modeling of JP-5 exposure.

PBPK/PD Modeling

The uptake and disposition of chemicals (drugs and pharmaceuticals) by living organisms, most notably humans, long has been one of the primary concerns of pharmacological research. The study of the dispersion of chemicals in animal bodies constitutes the field of pharmacokinetics—although, depending on the type of chemical studied, it has also been called toxicokinetics and biokinetics. For drug research, two parameters of the disposition of a drug are of special interest, namely the residence time of a compound in the body (most often reported as its half-life) and the (peak or average or both) target concentration—the amount of the drug that actually reaches the pharmacodynamic target site and is thus in principle available for therapeutic (inter)action. Because of the limited

*Bear in mind that all these modeling approaches, especially the PBPK paradigm, which relies heavily on the numerical solution (approximation) of sets of dependent differential equations, are subject to approximation and roundoff error. The larger the model description, the larger the total propagated error becomes for a given level of precision; to keep the level of error constant at a growing model size, the precision level must go up as a function of model size to a point at which this level of precision in combination with the limitations of the available hardware make use of a model impractical.

resources available for both detailed, time–course *in vivo* concentration studies, and for the computational effort needed, pharmacokinetic investigators traditionally described the pharmacokinetics of a compound with data-based compartmental models—they generally measure the time-course of the compound's concentration in a central compartment, normally blood, hypothesize a number of peripheral compartments of unknown size and chemical disposition, and fit an *n*-exponential model to the blood concentration versus time data, with *n* being the number of hypothesized compartments (*14*).

Although this approach generally works well if one has the data needed to fit the model to, some researchers clearly were dissatisfied with the apparent physiological meaninglessness of the compartments and compound rate constants that constituted the *n*-exponential model. As early as 1937 Teorell (*15,16*) described a modeling approach that rather than starting from arbitrary data, started with a functional but parsimonious physiological description of a living body. The approach divided the body into key tissue groups, with connecting blood flows providing inter-tissue transport, and membrane permeabilities and tissue partition coefficients describing the distribution of a compound over the different physiological compartments. In these original applications of physiologically based modeling, one of the major drawbacks was that to use the model, the set of linked differential equations actually had to be solved either analytically or by manually performed numerical approximation techniques. Consequently, this approach evolved only slowly during the next 40 years, until in the 1970s, major advances in computer hardware and software made possible for the first time the solution from the desktop of large sets of dependent differential equations needed to adequately describe a basic PBPK model within a reasonable amount of time.

So the basic difference between a classical pharmacokinetic model and a PBPK model is as follows: In the classical model not only the (compound) rate constants and concentrations but also the actual layout of the model (the topology of the model, i.e., the number and size of the compartments and the interconnections between them) are dependent on the experimental data (and thus on the chemical under study, the setup of the experiment, the route of exposure, and the dosing regimen). In the PBPK model, on the other hand, most if not all of the model topology as well as many of the rate constants and other chemical-dependent parameters can be determined a priori. The actual model topology can be determined by considering the animal under study and its physiology, the specific target for the chemical (drug) under study and its physiological location, and the specific metabolic and eliminatory pathways known for a drug; e.g., for a drug that is known not to pass the blood–brain barrier, a separate brain compartment is not needed for an adequate, parsimonious PBPK model. The chemical parameters needed for the adequate description of its pharmacokinetics can also be determined a priori; blood–air and tissue–blood partition coefficients can be measured *in vitro* by a number of different techniques [vial-headspace equilibration for volatiles (*17,18*), dialysis equilibration (*19*), or solid phase microextraction equilibration (*20*) for nonvolatiles]. Metabolic rate constants, for either Michaelis-Menten saturable degradation or for linear *n*th-order (normally first-order) kinetics can also be measured *in vitro* with, for example, liver homogenates for liver-based metabolism (*21*). When the model is complete, it is checked against actual experimental time–course data for the chemical and animal under study, and if correspondence is lacking, some of the parameters, within physiological and chemical constraints, can be adjusted to improve the model. Such a PBPK model has two major advantages over a classical model.

First, because it is based on the actual physiology of an animal, it not only empirically describes the concentration time–course in the compartment actually measured but also predicts the concentration time–course in all the other recognized compartments, notably the compartment holding the active target for a drug. The model in principle can even be refined to where it will accurately predict the concentration of a pharmacon at the specific target molecule.

Second, the model explicitly contains the physiologic description of an animal and the pertinent chemical parameters of the compound under interest, actual cross-species (or intra-species cross-lifestage) or cross-compound predictions are possible. It is this extrapolation possibility that makes PBPK (and/or PBPD) modeling such a promising tool in predictive toxicology.

So what does a basic PBPK model look like? A basic mammalian model, assuming exposure by inhalation to a volatile organic compound, is made up of the following compartments: lungs (the alveolar space part of them); lung blood, where the compound first enters the body proper; fat (adipose) tissue (this normally acts as a storage/sink compartment for lipophilic compounds, with back-delivery); richly perfused tissue (kidney, intestines, brain, liver)—liver is normally separated from the rest of the richly perfused tissue because it is the compartment where the metabolism is normally located; and poorly perfused tissue (mainly muscle and bone). Then there is the blood, which is usually divided into arterial blood, which leaves the lungs laden with a chemical and enters the tissues where it will redistribute it, and venous blood which leaves the tissues depleted with a chemical then returns to the lungs. The overall blood flow is described by cardiac output and by fractional blood flows entering the separate compartments. For other exposure routes, additional compartments can be defined such as a skin compartment for dermal uptake, a bulk bolus compartment for ip injection, or a gut compartment for uptake from food. Other elimination compartments, in addition to exhalation through the lungs, are the kidney for urinary excretion, the liver for biliary excretion, or the gut for direct desorption into fecal mass. See Figure 1 for a schematic overview of a basic PBPK model.

The model is linked mathematically by means of mass balance equations that describe movement of the compound from blood to tissue or vice-versa by considering the difference in concentration (or amount) of chemical in the arterial blood entering and the venous blood exiting a tissue compartment (*10*):

$$\frac{dA_i}{dt} = Q_i C_a - Q_i * (C_i / P_{b,i})$$

This equation indicates that the rate of change in amount of chemical in compartment i is a function of the blood flow to compartment Q_i times the concentration in the afferent blood C_a (what is coming in), minus the blood flow from the compartment (again Q_i) times the concentration in the efferent blood $C_i/P_{b,i}$, defined as the concentration in the tissue over the tissue–blood partition coefficient (what is going out); this description assumes that all compartments are internally well mixed, and that the efferent blood is in equilibrium with the tissue ($C_{vl} = C_i/P_{b,i}$). This type of basic model is called a flow-limited model. If the assumption of tissue–blood equilibrium is

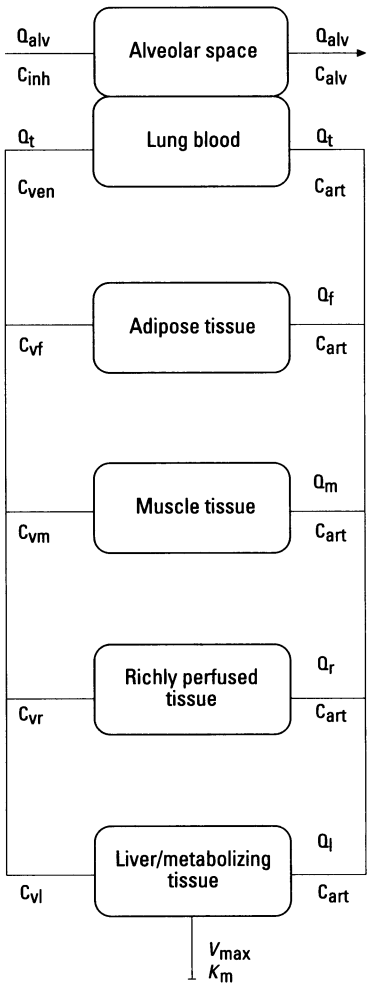

Figure 1. Schematic overview of the main compartments and blood and substance flows in a basic PBPK model. Abbreviations: Q_{alv}, alveolar ventilation; C_{inh}, concentration of compound in inhaled air; C_{alv}, concentration of compound in alveolar air; Q_t, cardiac output, or total blood flow rate; C_{art}, concentration of compound in arterial blood; Q_m, Q_f, Q_r, Q_l, blood flow rates to individual tissues ($Q_f + Q_m + Q_r + Q_l = Q_t$); C_{vf}, C_{vm}, C_{vr}, C_{vl}, concentrations of the compound in the efferent (venous) blood exiting the respective compartments; C_{ven}, concentration of compound in the venous blood entering the lungs; V_{max}, K_m, Michaelis–Menten kinetic constants. Figure adapted from Ramsey and Andersen (*22*).

not met for one or more compartments, we get a diffusion-limited model, in which two differential equations are needed for each compartment, one for the rate of change in the tissue proper and one for the rate of change in the tissue blood.

The major advantage of PBPK/PD modeling for mixture toxicity work is that, in principle, modeling the pharmacokinetics of a set of more than one compound is mathematically the same as modeling the kinetics of a single compound. Fortunately, the current state of computational technology in both hardware and software is such that models of such a complexity can easily be solved, even from the desktop, if a powerful enough computer (high-end desktop system, e.g., employing Intel P5 or Motorola PowerPC hardware or a workstation-type machine) is used. In the simplest case, it is assumed that the individual compounds do not interact at all. This leads to a model that is nothing more than a repetitious extension of a single compound model, where the only mixture toxicity integration performed is the possible adding of the multiple amounts at a target site. This assumption, however, while often quite useful in practice, violates theoretical considerations in almost all instances. Fortunately, a well-defined PBPK/PD model can incorporate known interactions between compounds at any of a number of levels; *a*) at the level of transport kinetics, where they could compete for free sites on transport proteins; *b*) at the level of metabolism and elimination, where they could either competitively or noncompetitively inhibit each other's turnover by one or more enzyme systems; and *c*) at the level of the pharmacodynamics, where they could competitively inhibit each other's binding to an active site at a target enzyme, or where one compound could display noncompetitive inhibition or stimulation of the action of another compound or even act as an antagonist. In some cases interactions of different compounds at disjoint target sites could even ultimately have interactive (synergistic or antagonistic) toxic effects. If known, all these types of interactions can in principle be provided for in a PBPK/PD model.

Quantitative Structure–Activity Relationships

Quantitative structure–activity relationships originated in its current form within the fields of agrochemistry and medicinal chemistry. Both disciplines needed tools to quantitatively correlate chemical structure with biological activity, namely pesticide action and specificity, and therapeutic potency, both to rationalize congener selection and to guide the synthesis of potentially more active or potent compounds. A number of researchers over the last 120 years have used the inherent notions of the QSAR discipline to explain or to model biological activity of sets of compounds, notably Crum-Brown and Frazer (*23*), Meyer (*24*), Overton (*25*) and Ferguson (*26*), but it was not until Hansch et al. published their seminal paper in *Nature* (*27*) that the field got a theoretical foundation and a solid start. Hansch et al. showed that for a set of congeneric compounds a statistical correlation could be found between a set of structural parameters and the concentration at which any chemical from this set elicited a certain set level of a specific biologic response (such as the LC_{50} concentration at which 50% of test organisms or cells die) that is of the following form:

$$\log(1/C) = a*\pi + b*\pi^2 + c*\varepsilon + d*S + e$$

where C is the concentration or dose at which a certain effect manifests itself at a certain level, π is a hydrophobicity term (later superseded by log K_{ow}) encoding for hydrophobic interactions between, for example, a molecule and a receptor site such as Van der Waals interactions, ε is an electronic term encoding for electronic interactions such as ionic binding or dipole interactions or hydrogen bonds, and S is a steric term related to bulk and shape. The quadratic term was introduced to empirically model the observed curvilinear relationship between log $1/C$ and hydrophobicity frequently encountered in single-dosing tests.

A number of rationalizations have been given for this basic QSAR equation, all of which explicitly or implicitly rely on the notions of linear free energy relationships. We will use the one given by Hermens (*28*) because we believe it to be one of the most illustrative rationalizations. Consider that the activity of a biologically active molecule can be described as being dependent on *a*) the probability Pr_1 that a molecule reaches its (proposed) target site; *b*) the probability Pr_2 that said molecule will successfully interact with this target; *c*) the dose, or external concentration C.

If we then assume that at a particular effect level the number of molecular events that has occurred C_t is constant, we can easily see that:

$$C_t = c * Pr_1 * Pr_2 * C = \text{constant}$$

which, when log-transformed, becomes:

$$\log(1/C) = c + \log Pr_1 + \log Pr_2.$$

Assuming then, without elaboration, that the hydrophobicity term (log K_{ow}) from the Hansch equation, being a partition coefficient, is a good indicator of log Pr_1, the probability of a chemical reaching a

normally lipophilic site, and that the electronic and steric terms correlate to log Pr_2, the actual interaction with the target site, for a set of congeneric molecules, the rationale behind the Hansch equation is clear.

Although QSAR analysis has been used extensively in the pharmaceutical field in the last 30 years, its application in toxicology has lagged, with the notable exception of the aquatic and related environmental toxicology areas. This apparent difference is probably because environmental toxicologists have to deal with such a large amount of different potential toxicants, and often on such short notice that the only way to provide information on the biological action of a chemical is to use predictive techniques. Both in the pharmaceutical/medicinal chemistry area of QSAR and in the aquatic toxicology QSAR field, important progress has been made during the last 15 years so that for large groups of chemicals, predictions about their pharmaceutical potency or their environmental effect (level) can reliably be made [for a recent overview, see either Hermens and Verhaar (*29*) or Verhaar (*30*)]—so much so that first and foremost in the United States but also in Europe, a substantial part of the environmental risk assessment process makes extensive use of QSAR predictions.

Some of the more important recent advances in QSAR are the development of more useful (user-friendly) and accurate quantum chemistry codes (programs) that can be used to calculate more adequate physicochemical descriptors than before (*31*), and can even be employed to calculate reaction kinetics data for certain types of toxicological interactions (*32*), the introduction of 3D-molecular modeling and 3D-QSAR methods that use actual 3-D information about a (set of) molecule(s) to predict receptor interaction and thereby biological activity (*33*), and more advanced correlation techniques such as partial least squares (PLS) (*34*), analysis or neural networks correlations. PLS analysis can start from a large set of potentially useful but mostly highly collinear descriptors and correlate these to one or more biological interactions in such a way that the collinearity between predictors is removed and the set of actual predictors is projected downward to only a few significant ones, thereby creating correlative models with a high predictability. Neural networks, on the other hand, can be trained on a descriptor and biological effect set in such a way that they will fit a closest nonlinear function to any correlation without having to specify the exact functional shape in advance.

Then what can QSAR do for PBPK modeling, especially in the application of PBPK modeling to mixtures? Consider that even in PBPK modeling of single compounds, for which a lot of experimental data are available or generated specifically for the modeling study of interest, a number of essential data frequently are not known or are of questionable value, and are often fitted a posteriori from comparison (either manual or by a curve-fitting analysis) with experimental pharmacokinetic data. In such cases predictive methods, especially if their applicability and reliablility are known, can fill in the blanks on an a priori basis. In the case of mixtures, especially large industrial mixtures, the chance that all pertinent chemical information needed for the modeling of the kinetics of all the components decreases exponentially with an increasing number of constituents of the mixture, and a priori predictive methods become almost imperative to the PBPK modeling effort. The problem is even worse when a modeling study must deal with mixtures of unknown or partially known composition. No experimental data can be used for constituents of unknown nature, and predictions of average chemical parameters of the mixture must be either based on a guess of the mixture's composition or on a carefully devised measurement of some key physicochemical property or property profile of the mixture.

The first and foremost property or actual set of properties needed for the successful development of a PBPK model are the tissue–blood, and blood–air partition coefficient for all involved compartments. These normally are derived either from (assumed) equilibrium *in vivo* situations or measured *in vitro*, either by a vial headspace equilibration method for volatile compounds or with dialysis methods or, more recently, SPME based methods for nonvolatiles. This set of properties is, within limits of chemical similarity, amenable to QSAR modeling of unknowns. The work of de Jongh and co-workers (*35*) showed that for a large set of diverse chemicals, in both rats and humans, the tissue–blood partition coefficients could be described by a general function of the compounds' log K_{ow}, of the following form:

$$P_{t,b} = \frac{f_{w,t} + f_{l,t} * K_{ow}^{a}}{f_{w,b} + f_{l,b} * K_{ow}^{b}} + c$$

where $P_{t,b}$ is the tissue-to-blood partition coefficient, $f_{w,t}$ is the fraction of water in the tissue, $f_{l,t}$ is the fraction of lipid in the tissue, $f_{w,b}$ is the fraction of water in the blood, $f_{l,b}$ is the fraction of lipid in the blood, a and b are collander-type exponents (these can be set to be the same also—they denote the similarity of the different lipids to octanol and to each other) and c is an adjustment factor (mainly) to provide for the effect of protein binding; this is most pronounced for low K_{ow} compounds.

The other area in which QSAR can be used for PBPK modeling input is in the prediction of metabolic rate constants and elimination constants. Since these processes depend on more types of specific interactions than just physicochemical partitioning behavior, the QSAR predictions for these parameters will be more specific to certain types of chemicals and organisms and will not be as generally applicable as the tissue–blood partition coefficient models. In fact, most of the QSAR applications in this particular field of PBPK parameter prediction must be developed as this field grows, since no specific models for these types of predictions have been published. Some general QSAR models for metabolic rate constants have been published [see, for example, (*36*)], but most of this research is still in its infancy.

Lumping Analysis

Unlike PBPK/PD modeling and QSAR analysis, lumping analysis is a discipline with which most toxicologists are probably not familiar. Therefore, it is discussed in a little more detail in this section.

Lumping Basics

Modeling the pharmacokinetics and pharmacodynamics of a complete complex mixture such as JP-5, a navy jet fuel, for predictive toxicology purposes, would be an extensive and perhaps impossible task in terms of both the time and complexity involved. A certain degree of simplification is needed to bring such a project into more realistic scope. This simplification can be accomplished through a process called lumping, in which similar components are grouped (lumped) into pseudocomponents. Whereas the actual mixture contains too many components to follow individually, a set of a few well-chosen or well-designed pseudocomponents could be modeled. A representative chemical, or perhaps a fictional average over the entire pseudocomponent, would then be chosen to substitute for each pseudocomponent to

yield the necessary chemical parameters such as tissue partition coefficients and diffusion rates; the PBPK modeling is then performed on these pseudocomponents.

The general concept of lumping involves taking a mixture of several chemicals and finding some relevant similarity that allows the grouping of certain chemicals. For example, in a mixture of eight chemicals (A_1, A_2, A_3, B_1, B_2, C_1, C_2, C_3), imagine that all of the As behave in a similar manner in the process of interest, and the same follows for the Bs and the Cs. Then the eight chemicals could be grouped into three new pseudocomponents A, B, and C, so that the mixture can be considered to contain only three relevant chemicals (Figure 2).

How the chemicals are lumped depends on the behavior of the chemicals being studied. In petroleum production processes where cracking or breaking apart the large molecules and subsequent distillation are the most important issues, the molecular weight or the boiling point might be the basis of grouping. In other situations, the octanol–water partition coefficient K_{ow} or key structural features might be more important. Regardless of the parameter used, a first-guess lumping scheme is generally based on intuition. In instances where these parameters are not known for some of the chemicals involved, their values can often be predicted by methods like QSAR.

Since the chemicals in a pseudocomponent have individual identities, they will not truly behave as a single chemical. Thus, lumping leads to a loss of information. In creating a lumping scheme, one must weigh the advantage of the ease of dealing with a smaller number of pseudocomponents against the disadvantage of the increased loss of information describing the system. A balance must be found between these two competing factors, and that balance will be unique for each situation.

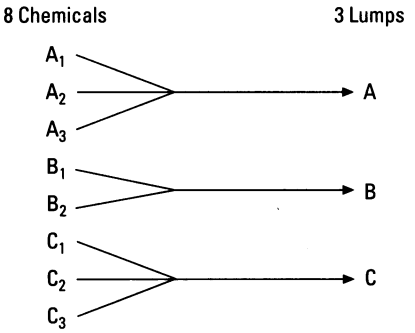

Figure 2. Lumping scheme to lump eight chemicals into three pseudocomponents.

Once the initial best-guess lumping scheme is formulated, in some cases certain mathematical methods exist to estimate the error introduced in transforming the individual chemicals to the lumped groups or from one scheme to another (*37*). This is discussed further in the section "Two General Cases." The scheme is then adjusted to limit the error as deemed necessary by the particular situation. The ideal situation would be one of invariant response, i.e., one in which the behavior of the system is not changed as the concentration ratio of chemicals within a given pseudocomponent is changed. If all the chemicals in a pseudocomponent behaved in exactly the same manner, this would be the case; however, this situation rarely if ever occurs.

Depending on the characteristics of the system of interest, different methods of lumping may be used. Some of these are

- A system can be described as either perfectly or imperfectly lumpable. If a single lumping scheme can be used to describe all subprocesses of the system, such as characterizing the inlet stream, the intermediate kinetics, and the outlet stream, then it is considered perfectly lumpable (*38*).
- A lumping scheme can be either proper, semiproper, or improper. If each chemical is put entirely into one pseudocomponent or another, then the scheme is proper. However, if 50% of chemical A is put in one pseudocomponent and 50% is put into a different pseudocomponent, then the scheme is either semiproper or improper (*38*). Most of the mathematics describing lumping deal strictly with proper schemes.
- Lumping can be performed in either a discrete or a continuous method. The example in Figure 2 was discrete: certain specific chemicals were placed into certain lumps. However, with a very large mixture of chemicals, a more continuous method might be used. An example of this would be to place all chemicals with molecular weights of 0 to 100 in pseudocomponent A, and those with molecular weights of 100 to 200 in pseudocomponent B. In this manner, the actual number or identity of the chemicals in the pseudocomponent may not be known. In a truly continuous lumping scheme, no discrete pseudocomponents exist. Rather, all the chemicals are described in a continuum [(*39*), see also the example in the next major section)].

As with any method of analysis, varying degrees of complexity exist. For example, although many of the processes deal strictly with reaction kinetics, some processes must also consider additional processes such as mass transfer. This is evident in the petroleum industry, where reaction kinetics may be increased by a catalyst. In this case, mass transfer is in the form of diffusion on a catalyst particle. This adds another level of complexity to the mathematics; and one that may become important in lumping analyses for PBPK modeling. A second complexity-enhancing aspect of lumping analysis involves precisely the reaction kinetics. The simplest of lumping schemes considers only first-order reaction kinetics; others also consider higher-order reactions. Sometimes higher-order reactions can be estimated as first-order, depending on the situation and the accuracy required.

Lumping Theory: Two General Cases

As discussed previously, several different levels of lumping exist; in fact, not all systems can be lumped in the same manner. However, two general cases exist. In the first case, all the chemicals involved in the system are well identified, and their behavior with respect to each other is known quantitatively (i.e., reaction kinetic rate information is available). A catalytic cracking process in the petroleum industry is an example of this case; this process will be illustrated later. In the second case, however, the mixture of chemicals involved is not explicitly defined, that is, the identities of the chemicals are not (exactly) known, and their behavior has not been described quantitatively or even qualitatively.

Lumping analysis was first introduced by Kuo and Wei (*37*) and Wei and Kuo (*38*) in the 1960s. They presented a mathematical approach to lumping a well-defined mixture of chemicals. To give a better understanding of this mathematical process of lumping analysis, a simplification of an example illustrated in their work is given here, along with the basic mathematics needed to describe it. More specific and complete information is provided in their original papers.

For a single reaction: $x \xrightarrow{k} y$, the reacting species is x, and the rate of reaction is k. With reversible reactions and multiple interrelated reactions, a matrix format helps to reduce the number of equations and simplify the mathematics. In case 1, these reactants and products, as well as the reaction kinetics that link all species, are known. Consider the linear reaction system involving three species A_1, A_2, and A_3, described as follows (Figure 3).

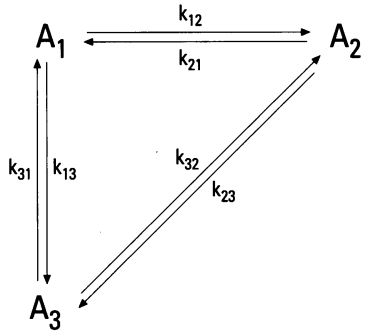

Figure 3. Linear, reversible reaction system of three species.

For this system, the actual species composition matrix (a matrix that includes all species involved, both reactants and products) is

$$a = \begin{pmatrix} a_1 \\ a_2 \\ a_3 \end{pmatrix}$$

where a_i is the fractional composition of species A_i. If we seek to lump together a_1 and a_2 (because of similarities in behavior) into $\hat{a}_1$ such that $a_1 + a_2 = \hat{a}_1$ (and $a_3 = \hat{a}_2$), then the lumped composition matrix is

$$\hat{a} = \begin{pmatrix} \hat{a}_1 \\ \hat{a}_2 \end{pmatrix}$$

The lumped reaction system between $\hat{A}_1$ and $\hat{A}_2$ could be illustrated as:

$$\hat{A}_1 \underset{\hat{k}_{21}}{\overset{\hat{k}_{12}}{\rightleftarrows}} \hat{A}_2$$

The lumping matrix M is used to mathematically relate the lumped species matrix to the unlumped species matrix. For the example, the lumping matrix is defined by

$$\hat{a} = M * a$$

so that

$$M = \begin{pmatrix} 1 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

Differential equations are used to mathematically describe the concentrations of the chemical species as they change with time. In a first-order reaction, the derivative of the concentration is expressed as the product of the concentration itself and a reaction rate, as shown:

$$\frac{da}{dt} = -ka$$

$$\frac{d\hat{a}}{dt} = -\hat{k}\hat{a}$$

Since the concentrations of the chemical species are expressed in matrix form, the reaction rates must also be expressed in matrix form. For the original species, the unlumped rate matrix is,

$$K = \begin{pmatrix} \sum k_{i1} & -k_{12} & -k_{13} \\ -k_{21} & \sum k_{i2} & -k_{23} \\ -k_{31} & -k_{32} & \sum k_{i3} \end{pmatrix}$$

and the lumped rate matrix for the lumped species is

$$\hat{K} = \begin{pmatrix} \hat{k}_{21} & -\hat{k}_{12} \\ -\hat{k}_{21} & \hat{k}_{12} \end{pmatrix}$$

Other matrix manipulations take off from this point, and the papers by Wei and Kuo (*38*) and Kuo and Wei (*37*) illustrate other valid mathematics that apply to this example. Even from the limited mathematics presented here, it is evident that much has been gained by lumping. Originally, three different species had to be considered, along with the three reversible reactions that connected them. However, with the new pseudocomponents, only two species have to be considered, and now there is only one reversible reaction. Thus, from lumping, the matrices to be manipulated are considerably smaller and less subsequent computation will be required. Although this was a very simple example, it is apparent that with larger systems (those with five or ten or hundreds of components) the simplification could be immense.

A real-world example of such a well-defined lumping system is the Mobil 10-lump model, which was developed for catalytic cracking processes in the petroleum industry. This model is illustrated in Figure 4.

In this model, the many petroleum compounds involved have been lumped into ten groups according to similarities in molecular structure and boiling point. Catalytic cracking is the decomposition of larger molecules into smaller molecules to achieve a more useful product, gasoline. However, only a fraction of these smaller molecules comprises the gasoline lump; those molecules even smaller than those in gasoline are grouped in the C lump. Average reaction kinetics have been determined for each of the reaction arrows shown in the diagram, so this system can be mathematically described in a manner similar to the matrix example described previously (*13*).

The Mobil 10-lump scheme has been shown in the petroleum industry to be the most effective for the situation in which it is used, but, of course, an infinite number of possible schemes exist in theory. The key to lumping is to choose the lumping scheme that provides simplicity while maintaining a level of accuracy deemed necessary for the particular situation. For systems that fall into case 1, the well-defined case, there exists a mathematical means of determining the error introduced as additional groups are lumped together. Known as cluster analysis, this method is based on a set of statistical equations (*13*). The error introduced with each additional reduction in system complexity (i.e., in going from 10 to 9 to 8 to 7 lumps) can be calculated, and the point at which the error exceeds an arbitrary acceptable level can be determined.

Systems for which most or all of the relevant properties are unknown (case 2) are more difficult to describe mathematically. Information on the chemical species present and their behavior is lacking, so a different approach must be taken in analyzing this type of system. Informed assumptions form a basis in this type of analysis. For simple mixtures containing a relatively small number of known chemicals (e.g., less than 20) for which parameter values are unknown, a QSAR method can be used to estimate those values (see the QSAR section) and a discrete lumping method can be performed over the estimated parameters. For most complex mixtures such as gasoline or JP-5 (a naval jet fuel), the identity and concentration of each component may be unknown; even the total number of major and minor components might not be readily available. Thus, discrete lumping and classical QSAR methods cannot be

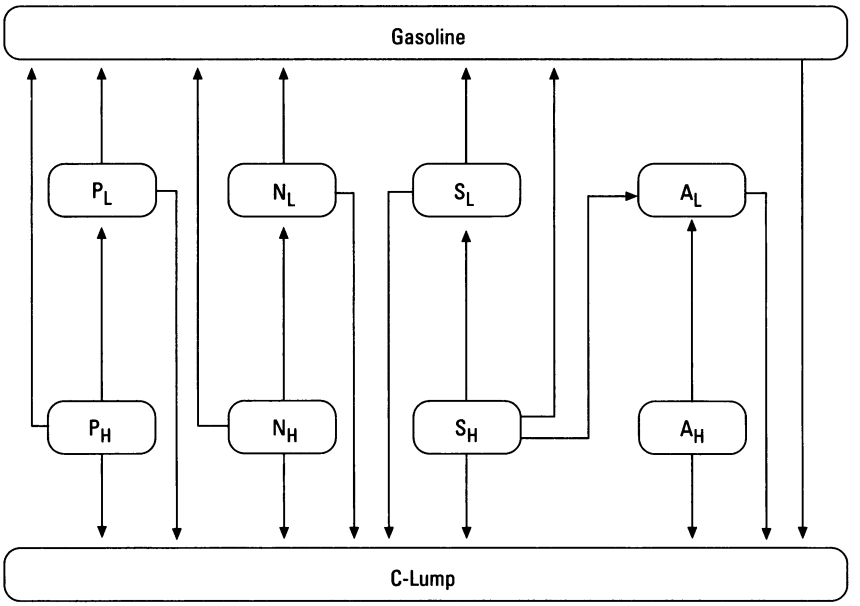

Figure 4. Schematic of the Mobil 10-lump scheme. Each lump reacts to yield the product pointed to by the arrow. [Redrawn from Coxson and Bischoff (*13*)]. The ten lump species are P_H, wt% paraffinic molecules, 650° F+; N_H, wt.% naphthenic molecules, 650° F+; S_H, wt% aromatic side chains, 650° F+; A_H, wt % carbon atoms among aromatic rings, 650° F+; P_L, wt % paraffinic molecules, 430–650° F; N_L = wt % naphthenic molecules, 430–650° F; S_L, wt % aromatic side chains, 430–650° F; A_L, wt % carbon atoms among aromatic rings, 430–650° F; G, gasoline lump (C5–430° F); C, C–lump (C1 to C4 + coke).

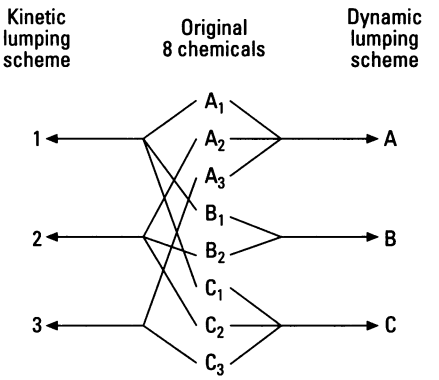

Figure 5. Schematic of imperfect lumping.

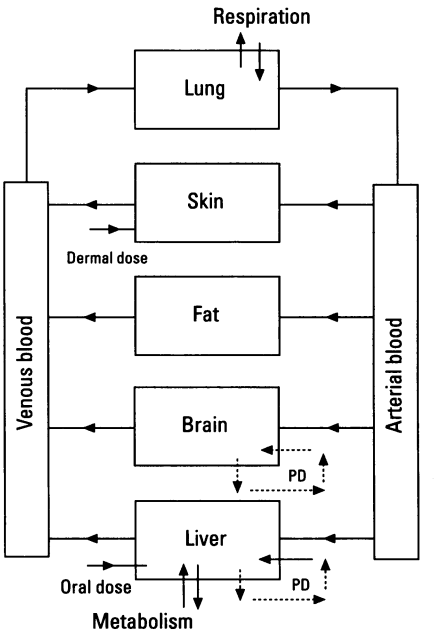

Figure 6. General compartmental diagram of imperfect lumping scheme incorporated into PBPK/PD modeling.

used. Instead, a feasible approach is to use available information on the mixture as a whole (i.e., boiling points of fractions or the boiling range and behavior) in QSAR models to estimate continuous parameter value distributions that then form the basis for a continuous or semicontinuous lumping scheme. It may also be possible to use QSAR estimates in the cluster analysis method to provide guidance in developing the lumping scheme.

For example, the naval jet fuel JP-5 is composed of hundreds of compounds, but the exact composition has not been determined and actually can vary considerably depending on origin of crude employed, specific method to formulate the final product, and actual provider/refiner. However, general knowledge about the composition, such as general composition groups, is available. Using this information in conjunction with certain known properties of JP-5 (such as boiling and freezing points), a good guess can be made for the composition of JP-5 (see the section on Integration). Using this information, the components can be lumped according to known or estimated properties that might dictate the behaviour of interest of the system (such as lumping by K_{ow} to analyze distribution within a physiological system).

Frequently, a more continuous approach to lumping (such as in the previous example) is helpful with ill-described systems. In this way, lumps can be made, but the exact composition of each lump need not be known. Rather, using information available for a particular group of compounds, average parameters may be assigned to the lump.

Application of Lumping Analysis to PBPK Modeling

Using lumping analysis in a PBPK model of a complex mixture involves several aspects not present in traditional petroleum lumping analyses. For example, a single lumping scheme may not suffice for the entire model, which implies a need to use imperfect lumping. A likely scenario is that one lumping scheme may be appropriate for the pharmacokinetics of distribution of the mixture in the body, while a second scheme may be necessary to describe the pharmacodynamics of the chemicals involved (Figure 5).

This improper lumping approach is also illustrated in Figure 6 as a very general compartmental diagram. Scheme 1, the pharmacokinetic scheme (which lumps those chemicals with similar pharmacokinetic fate), is designated by the solid arrows, and scheme 2, the dynamic scheme (which lumps those chemicals with similar pharmacodynamic fate), is designated by the dashed arrows. The relumping between schemes is done computationally.

Another issue to consider in PBPK modeling of complex mixtures is interactions between the lumps. Interactions between lumps can be modeled just as they are for interactions between single chemicals. Interactions can be additive, synergistic, or antagonistic (*40*). No matter what type of interactions exist, each lump is treated as a single chemical. Thus, the lumping scheme must be chosen in such a way that interactions between lumps can be described in this manner and that significant interactions do not occur within a pseudocomponent.

As an example, a general procedure addressing the PBPK/PD modeling of a general complex mixture, here the naval jet fuel JP-5, is outlined below.

- Determine a first-pass proper lumping scheme: JP-5 is composed of many different chemicals; thus, the initial analysis will be a semicontinuous form of lumping. That is, based on our knowledge of the composition of JP-5, we will separate the major or significant components into discrete lumps. If it becomes evident at a later date that a continuous analysis would be more appropriate, it can be further investigated at that time.
- Formulate the PBPK/PD model, including reaction kinetics and mass transfer. For the reaction kinetics, it may be much easier to assume first-order kinetics; however, Michaelis–Menten kinetics are often implicated in the metabolism of chemicals. With initial models first-order kinetics may be assumed. However, more accuracy can be incorporated into subsequent models. The mathematics required to incorporate the mass transfer inherent in a PBPK/PD model can be based either on simple partition coefficient schemes, such as that shown later in the JP-5 example, or, in more complex cases, on those used with diffusion on a catalyst particle. Several papers in the literature address this issue in the petroleum industry (*38,41*).
- Evaluate performance and consider the addition of complexities: As with any situation, the initial PBPK/PD model is a simplified version of reality. To increase the accuracy of the model, interactions in the reaction kinetics and an improper lumping scheme may be included.

Integration: An Example Using JP-5, a Navy Jet Fuel

Introduction

JP-5 is a jet fuel of the kerosene type. It can be formulated from both crude petroleum and shale or from other synthetic crudes. It is intended to be used in Navy jet fighters, mainly deployed from aircraft carriers, and thus is subject to certain specifications to ensure its suitability for storage and use on board marine vessels. The major requirement specific to JP-5 is a rather high limit flashpoint of 60°C. This requirement immediately points to the main difference between JP-5 and JP-4, a wide-cut kerosene-type jet fuel that is almost identical to commercial Jet-A and heating fuels 1 and 2 (the difference is the narrower and higher boiling range of JP-5 vs JP-4). JP-4 has a cut range of approximately 120 to 280°C, whereas JP-5 has a cut range of approximately 200 to 290°C. Consequently, JP-4 and other wide-cut kerosenes contain hydrocarbons ranging from C4 to C16, while JP-5 and similar narrow-cut kerosenes contain hydrocarbons in the C9 to C18 range.

Composition

Since JP-5 is a product that is formulated based on certain physical and physicochemical properties rather than on actual chemical makeup, considerable variation exists in actual JP-5 composition, caused mainly by differences in crude feed material and refinery-specific methods used in creating a product that adheres to the required specifications. Roughly speaking, a JP-5 sample will consist of petroleum-derived hydrocarbons, generally some 100 to 300 different constituents, plus a number of property-enhancing additives that constitute a very minor amount on a weight/weight basis. Most if not all JP-5 stocks can be considered to have roughly the following composition, according to sources pertaining to jet fuel composition (*42–45*).

Hydrocarbon fraction

- Paraffins (alkanes)
  30 to 40% (6–8% *n*-paraffins; C9–C18)
- Olefins (alkenes)
  1 to 4%
- Aromatics
  ± 20% (almost no PAHs; highly substituted)
- Naphthenes (cycloalkanes and cycloalkenes)
  30 to 50%
- Nitrogen compounds
  < 0.3%
- Sulfur compounds
  ± 0.1% (mostly disulfides R–S–S–R)

Known additives

- Diethyleneglycol monomethylether (anti-icing agent)
- Dicarboxylic acids (anti-corrosives)
- Hindered phenols (anti-oxidants)
- *N,N'*-disalicylidene-1,2-propanediamine (metal chelator)

A partial compositional breakdown of JP-5 is also available (*46*). This describes about 36% of the composition of JP-5 on a weight basis. This partial composition information can be used to estimate a chemical profile for JP-5 as a whole. See Table 1 for an overview of the known composition of JP-5. Figure 7 shows the distribution of mole fractions over log K_{ow} windows based on the composition presented in Table 1 (light bars) and based on a hypothetical composition assuming that the unaccounted for compounds follow hydrophobicity profiles similar to that of the known compounds.

Systems To Be Modeled

Since JP-5 can be both a potential human health hazard (as in acute or chronic exposure of petroleum industry workers, fuel and airplane technicians, and aviators to jet fuel fumes or liquid present in their normal working environment (*47,45,48*), as well as members of the general public following major spills, from continuous leaks or evaporative loss from contaminated sites), and an environmental health hazard (as air, water, or soil pollutant), it should be decided beforehand what biological system will be modeled and why.

Since it is more likely that JP-5 poses a health threat to workers who are occupationally exposed to it than that JP-5 will feature in a major environmental contamination, it appears that modeling human health effects would be the most appropriate action. If so, it can safely be assumed that human exposure will in most instances be inhalatory in nature—ingestion of appreciable amounts of JP-5 is an unlikely occurrence other than possibly in calamity situations. Dermal exposure long and severe enough to pose a significant threat to human health is fairly unlikely but could in principle be experienced by maintenance and fuel delivery workers both in processing, transporting, and shipping enterprises and by maintenance personnel for navy jet fighter assemblies. Therefore, our first choice is to try to model distribution and effects following acute and chronic inhalatory exposure, with transdermal exposure situations a close second. An added benefit of including dermal exposure routes in a PBPK model-based risk assessment is that from target-effect levels determined or calculated for, for example, inhalatory exposure, coupled with reliable data on transdermal transport of a toxicant, external effect levels can be accurately determined for this exposure route. The same is true for other exposure routes, hence the ability of good PBPK models to assist in route-to-route extrapolation.

Table 1. Partial composition of JP-5, based on American Petroleum Institute (*46*).

Compound	Molar mass	JP-5, %	Mole fraction, %	ClogP
Octane	114	0.12	0.082135	4.93
Nonane	128	0.38	0.292035	5.455
Decane	142	1.79	1.526101	5.984
Undecane	156	3.95	3.699675	6.513
Dodecane	170	3.94	4.021491	7.04
Tridecane	184	3.45	3.81135	7.571
Tetradecane	198	2.72	3.233523	8.1
Pentadecane	212	1.67	2.125662	8.63
Hexadecane	226	1.07	1.451891	9.16
Heptadecane	240	0.12	0.172916	9.69
3-Methyloctane	128	0.07	0.053796	5.325
2-Methylundecane	170	1.39	1.418749	6.912
2,6-Dimethylundecane	184	2	2.209478	7.311
1,3,5-Trimethylcyclohexane	126	0.09	0.068086	4.911
1,1,3-Trimethylcyclohexane	126	0.05	0.037825	4.911
n-Butylcyclohexane	142	0.9	0.767313	5.46
Heptylcyclohexane	184	0.99	1.093692	7.05
m-Xylene	106	0.13	0.082735	3.14
o-Xylene	106	0.09	0.057278	3.09
1,2,4-Trimethylbenzene	120	0.37	0.266578	3.589
1,3-Diethylbenzene	134	0.61	0.490768	4.198
1,4-Diethylbenzene	134	0.77	0.619494	4.2
1,2,3,4-Tetramethylbenzene	134	1.48	1.190717	3.98
1-Ethylpropylbenzene	148	1.16	1.03077	4.73
1,2,4-Triethylbenzene	162	0.72	0.700309	5.18
Phenylcyclohexane	160	0.82	0.787727	4.76
1-*t*-Butyl-3,4,5-trimethylbenzene	176	0.24	0.25361	5.37
n-Heptylbenzene	176	0.27	0.285311	5.82
n-Octylbenzene	190	0.78	0.889795	6.34
Naphthalene	128	0.57	0.438053	3.316
2-Methylnaphthalene	142	1.38	1.176547	3.815
1-Methylnaphthalene	142	1.44	1.227701	3.815
2,6-Dimethylnaphthalene	156	1.12	1.049022	4.314
Biphenyl	154	0.7	0.647233	4.03
1-Ethylnaphthalene	156	0.32	0.299721	4.34
2,3-Dimethylnaphthalene	156	0.46	0.430848	4.26
Tridecene	182	0.45	0.491729	7.09

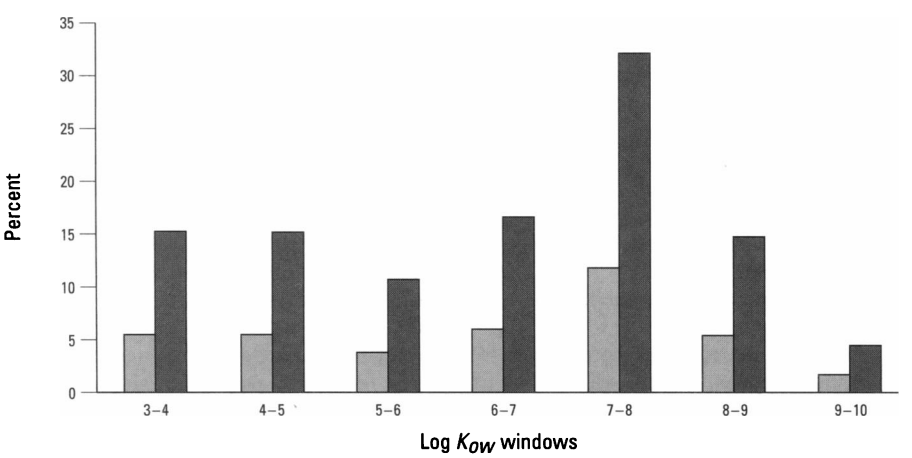

Figure 7. Distribution of tentative JP-5 mole fractions over 1 log unit log K_{ow} windows from 3 to 10. Light-colored bars are for percentages based on the fact that the JP-5 composition on which this exercise was based represented some 36% of its total mass; dark-colored bars are based on the assumption that the missing 64% is proportionally distributed over the recognized windows.

What Data/Parameters Are Needed To Adequately Model the PBPK Behavior of JP-5 after Inhalatory Exposure?

Since we are interested, at least initially, in the modeling of JP-5 after inhalatory exposure, the first thing needed is either a volatility profile of JP-5 or (more likely) data on the actual constitution of JP-5 vapor. This could probably be obtained by analyzing the constitution of JP-5 headspace vapor instead of liquid JP-5 samples, wherever in subsequent sections mention is made of actual measurements on JP-5 material.

The next set of data needed is an overview of PBPK-relevant (air–blood, blood–tissue) partition coefficients for JP-5 constituents. Another set of data called for consists of (qualitative or quantitative) information on biotransformation (metabolism) of JP-5 constituents.

Finally, effect parameters are needed to model specific toxicological end-points such as reactivity parameters for olefins that might be present in JP-5 and carcinogenicity parameters for possible residual PAHs.

Known Toxicological Effects of JP-5

Expected but not Substantiated Effects. Some research has been done on the possible toxicological effects of human and animal exposure to JP-5; human exposure has been focused mainly on inhalatory effects from JP-5 vapors, whereas animal studies (rats and mice) have been performed for inhalatory exposure as well as gavage dosing, dermal application, and intravenous administration (*48*). The main conclusions that can be drawn from the results presented for these studies are as follows:

According to a mouse dermal application study (*49*) as well as a *Salmonella typhimurium* mutagenicity test (*49*), JP-5 seems to be noncarcinogenic. This is somewhat surprising given the fact that a similar diesel fuel marine (DFM) is carcinogenic. The difference might be that, unlike DFM, JP-5 contains almost no polycyclic aromatic hydrocarbons (PAHs) (*48*).

JP-5 and other kerosene products have an animal-specific renal toxicity to the male rat. Since this effect is directed to a unique rat protein, namely $\alpha_2\mu$-globulin, which is pheromone-related, it is highly unlikely that JP-5 will exhibit such renal toxicity to species other than rat, including humans (*50,44*).

Although not reported, toxic effects could in principle be expected from the known additives to JP-5, since all additives are known toxicants (*42,49,48*). It should be noted here that the additives are present in very small quantities and that they are

all highly nonvolatile. It therefore seems highly unlikely that effects would be seen after inhalation exposure to JP-5 vapors. Exposure to these additives is to be anticipated after transdermal or oral exposition, but so far no indication for the subsequent occurrence of toxic effects has been found in gavage or dermal application animal studies (*48,44*).

Target Organ Effects. Studies of JP-5 target organ effects have included the hematopoietic system. In a series of studies conducted at Wright-Patterson Air Force Base, Gaworski, MacEwen, Vernot, and co-workers exposed beagle dogs, F344 rats, and C57BL/6 mice for 90 days to JP-5 fuel vapors from either petroleum or shale oil sources [summarized in Gaworksi et al. (*51*) and MacEwen and Vernot (*52*)]. Hematocrit, hemoglobin, total erythrocytes, total leukocytes, differential counts, mean corpuscular volume, and mean corpuscular hemoglobin concentrations were determined. Beagle dog blood parameters remained within normal limits, although a slight erythrocyte osmotic fragility was observed after 90 days exposure at 750 mg m^{-3}. No other significant changes were observed. Some fluctuations of erythrocyte levels were observed in rats, accompanied by slight changes in hematocrit and hemoglobin. No hematological effects were observed in C57BL/6 mice. Pathological studies of F344 rats and C57BL/6 mice showed no dose-related increases in leukemia in rats and no significant exposure-related effects in mice.

Liver toxicity of JP-5 has been studied in experimental rodents. Parker et al. (*53*) administered oral doses of JP-5 to male Sprague-Dawley rats and observed an oral LD_{50} of > 60 ml kg^{-1}. Animals that died during the 40-day holding period had swollen, mottled livers and histopathologic evaluation of the survivors indicated hepatic periportal fatty changes. Similar studies using single oral doses of JP-5 followed by sacrifices over the next three days showed a clear pattern of liver injury accompanied by increased serum lactate dehydrogenase, glutamic-transaminase, and glutamic–pyruvate transaminase levels (*53,44,54*). Inhalation exposures of beagle dogs, F344 rats, and C57BL/6 mice were conducted as part of the Wright-Patterson studies (*55*). Immediately after exposure ended, the dogs exhibited diffuse mild swellings and clouding of hepatocytes which later were described as containing excess glycogen. Beagle dog exposures to 750 mg m^{-3} resulted in increased liver weights, increased liver/body weight ratios, and decreased glutamic-pyruvate transaminase levels. Rats showed no histopathology or serum enzyme changes immediately after exposure, but after 19 to 21 months there was mild hepatic hyperplasia. Exposed female mice in the same studies showed focal fatty changes in hepatocytes that stained positive for fat and mild diffuse cytoplasmic vacuolization that was negative for fat and glycogen only in the 750 mg m^{-3} exposure group. These changes resolved by 19 to 21 months post-exposure. In a study involving intermittent exposures (6 hr/day, 5 days/week for 6 weeks) of rats to 1100 or 1600 mg m^{-3} of JP-5, Bogo et al. (*54*) reported no adverse effects in the liver and no change in serum enzyme levels.

Central nervous system (CNS) toxicity, generally classified under narcosis or general anaesthesia, is common to all volatile hydrocarbons (*56,57*) and is similar to (or the same as) the narcosis effects of, for example, diethyl ether, nitrous oxide, or halothanes (*58*). For example, Davies (*59*) described an incident in which a jet pilot was exposed to JP-4 vapors from a leaking fuel line during take off. He reported feeling groggy and weak and landed immediately. He exhibited a staggering gait, slurred speech, and signs of early anesthesia that were confirmed on physical examination. The pilot reported that he did not feel normal 36 hr after the event but appeared in good condition during the next few days and upon reexamination 5 months later. Estimated exposure concentration was 24,000 to 56,000 mg m^{-3} for a period of approximately 20 min.

It is assumed that this toxic effect is not compound-specific and is linked to the dissolution of the xenobiotic molecules in the cell membrane of (a.o.) nerve cells, thereby disrupting their normal operation and leading to depressed nerve cell action. There is a direct connection between a compound's hydrophobicity (expressed as its log K_{ow}) and its efficacy as a general anesthetic (*58*). The achievable depth of anesthesia is related to the minimum alveolar concentration necessary to produce anesthesia. The rate of anaesthetic onset is related to the blood/gas partition coefficient; for low ratios, onset is rapid and for high ratios onset is slow.

Chronic effects, following either prolonged (semi)continuous exposure to low or intermediate doses (concentrations) of JP-5 or acute exposure to extremely high concentrations, can tentatively be classified as Psycho-organic Solvent Syndrome (*60*), a condition characterized by general feelings of depression and inadequacy in the early stages and by concentration problems, loss of memory, impairment of mental abilities, and possibly dementia on prolonged exposure (several years or more). This condition seems to be slowly reversible in the initial (depression-like) stages but is only partly or nonreversible after onset of the more serious symptoms. The causative mechanism of this toxicological syndrome is not known, but one suggestion is that it might be linked to progressive demyelinization of nerve cell axons caused by the exposure to solvents. This effect might then also be linked to a compound's hydrophobicity, indicating its solvation potential to fatty molecules. However, attribution of a chronic neurological syndrome to solvents or other organic chemical exposures has been controversial (*61*).

Data on chronic human exposure to jet fuels is limited. Knave and co-workers reported on two groups of aircraft factory workers exposed to jet fuels in the course of their daily activities (*62–66*). No controls were available for this study. Reported symptoms of exposure included dizziness, headache, nausea, palpitations, and feeling of pressure on the chest during acute exposures at work. Chronic symptoms included neurasthenia, psychasthenia, and symptoms indicative of polyneuropathy. Clinical evaluation of nerve conduction velocity and vibration threshold revealed no remarkable findings. However, analysis of these exposed workers and reference groups by the authors led them to conclude that the jet fuel-exposed workers demonstrated a higher than expected prevalence of these symptoms. In a study designed to obtain more information on chronic neurological effects of jet fuels, Knave et al. (*64*) conducted a second study of workers in a jet engine factory. From this study they concluded that there was a higher prevalence of neurasthenic symptoms, greater irregularity of complex reaction time test performance results, greater performance decrement over time for simple reaction time test performance, and poorer performance in perceptual speed tasks.

In light of the above toxicological profile, it is concluded that our first aim in modeling the toxicity and toxicokinetics of JP-5 should be modeling of acute intoxication through noncompound-specific narcosis. The effect is assumed to be purely toxicokinetics driven and well correlated to the final concentration of compound in a lipoid tissue, presumably the nerve cell

membrane. This dose metric in principle is amenable to PBPK modeling. Constructing such a model should therefore be the first stage in the risk assessment of JP-5, according to the approach described here.

How Do We Obtain the Necessary Pharmacokinetic Parameters and Toxicological Information?

Partition Coefficients. It appears that partition coefficients constitute essential parameters for the modeling of distribution, as well as distribution-linked toxic effects of JP-5. Therefore, it is important to gain information on the hydrophobicity profile of JP-5 and/or its constituents.

Approach 1: The noncompound-specific approach. Since most if not all toxic effects expected from exposure to JP-5 are noncompound-specific, it should not be necessary to identify and quantify individual components of JP-5 (vapor) to model the distribution and effects of JP-5 in humans [see, for example, Verhaar et al. (*67*)]. A nonspecific hydrophobicity profile could possibly suffice. To this end, an assay was recently devised by Hermens and Verbruggen (*68*) based on work by Verhaar et al. (*67*) in which a chemical mixture is separated by C_{18}-reversed phase HPLC and the effluent is pooled into equally spaced, log K_{ow}-related windows. The total amount of compounds present in any one pool, on a molar basis, is then quantified by vapor pressure osmometry. The outcome of this procedure is a profile of a mixture that indicates, in window averages of 1 log unit, the mole fractions of a mixture in each window. These windows, together with the data on mole fraction contained in them, can then be used as a set of surrogate components of JP-5, about which all necessary information is known, namely amount, (average) hydrophobicity, and toxic action.

Approach 2. Compound-specific approaches. If a compound-specific approach is preferred, JP-5 samples could be characterized using gas chromatography (GC) or gas chromatography–mass spectography (GC–MS) techniques, and the major components, up to a total w/w percentage of ideally 60 to 95%, identified and quantified. Log K_{ow} values could then be either retrieved or generated for each of the identified components and the behaviour of JP-5 modeled as the joint (additive) action of all these individual components.

Alternatively, the approach mentioned in the Composition section, where JP-5 is described based on partial composition data, assuming the unknown fraction is similar in hydrophobicity profile to the known fraction, could be used to generate a compound-specific partition coefficient profile of JP-5.

Additionally, knowledge or partial knowledge about the composition of JP-5 would allow a functional lumping analysis, based on the hydrocarbon block method (*69–71*), thereby greatly reducing the complexity of the subsequent modeling process (not unlike when using the surrogate components). The hydrocarbon block method is an approach in which based on knowledge of the (preferably exact) composition of a hydrocarbon mixture, one assigns each constituent to a certain pseudocompound based on its hydrophobicity (log K_{ow}). The pseudocompounds are normally chosen to represent consecutive 1 log unit K_{ow} windows.

Physiological Partition Coefficients. If a compound-specific approach is taken, the literature could be searched for physiological partition coefficients for individual components. For those components for which no such partition coefficients are available, or for the surrogate components if the noncompound-specific approach is taken, the formulae developed by De Jongh et al. (*35*) can be used to predict the different physiological partition coefficients from log K_{ow} values and physical parameters for the appropriate compartments. This is a very elegant and valid approach for the prediction of tissue partition coefficients.

Vapor Composition. Since our main interest is the modeling of effects from inhalation exposure to JP-5 fumes, we should be concerned with the composition of JP-5 vapor instead of JP-5 liquid bulk, which will show considerable differences. Bishop et al. (*45*) showed that the average molar mass of the liquid bulk of a JP-4 sample was 125 g, whereas the molar mass of the associated headspace vapor was 85 g. Note that the observed difference will be less pronounced for JP-5 because of its narrower overall boiling range with a higher low end. This specific problem could be tackled in two ways. First, for any of the analytical methods described above in the Partition Coefficients section, the sample to be analyzed could be taken from a controlled JP-5 headspace instead of from JP-5 bulk liquid. Alternatively, vapor pressures or Henry's law constants could be predicted from hydrophobicity profiles as generated by the Verbruggen method (*68*). The vapor pressure prediction procedures have been developed and partially tested, but have yet to be published (Verbruggen, personal communication).

Biotransformation (Metabolism). There are known biotransformation pathways for a number of identified or suspected constituents of JP-5. Examples are toluene (converted to nontoxic benzoic acid, then converted to hippuric acid and excreted) and styrene (converted to mandelic acid, then converted to phenylglyoxylic acid). Hexane, which is converted to the highly neurotoxic 2,5-hexanedione, is not expected in JP-5, although it is a constituent of JP-4 and gasoline. Specific metabolic pathways for other (higher) *n*-alkanes and branched alkanes undoubtedly exist and a literature search could be done to uncover the primary metabolic routes for paraffinic, olefinic, aromatic, and naphthenic jet fuel constituents.

Again, this aspect can be approached in different ways. If a noncompound-specific approach is taken, a generalized metabolic pathway may be indicated for each of a number of classes of chemicals (namely *N*-alkanes, branched alkanes, alkenes, alkyl-substituted benzenes, alkenyl-substituted benzenes, naphthenes, and PAHs); most metabolites will be detoxifying, meaning that they will be less toxic, more water-soluble, and more easily excreted than their parent compounds. It should be kept in mind, however, that exceptions do exist. For these metabolic pathways, generalized rate constants could be used; these rate constants could either be predicted, based on QSAR models, or taken as literature values for appropriate example compounds if available. Distribution of compound classes over high performance liquid chromatography (HPLC) pools (surrogate components) could be considered uniform (zeroth order approximation) or could be predicted from nuclear magnetic resonance (NMR) or IR profiles measured on the individual pools.

If a compound-specific approach is taken (either exhaustive or model-compound-based), metabolic pathways for each of these compounds plus appropriate rate constants could be extracted from the literature or databases or, if unavailable, predicted using QSAR methods. A literature search for published rate constants would then have to be carried out.

Reactivity. Since reactivity is expected to be a minor if not negligible, factor in JP-5 toxicity, a sophisticated approach does not appear necessary except, perhaps, for metabolism. Some nonspecific reactivity might in principle be expected due to the

presence of unsaturated aliphatic moieties, especially if the unsaturations are conjugated with other aliphatic or aromatic unsaturations. Assuming a surrogate component approach, it might be enough just to determine the amount of olefins present in any single surrogate component (HPLC pool; log K_{ow} window); a feasible way to achieve this would be by running an NMR or IR profile of each HPLC pool.

Carcinogenicity. The same reasoning as for reactivity also holds for carcinogenicity. JP-5 is tentatively classified as noncarcinogenic. If, however, some attention is given to the modeling of possible carcinogenic behavior of JP-5, one consideration might be to estimate the fraction of aromatic moieties in each surrogate component, to model tissue distribution and retention of possible carcinogens. Again this could be achieved by running an NMR or IR profile of each HPLC pool. Note, however, that the only real carcinogen from this group, namely unsubstituted benzene, is not present in any significant amount in JP-5.

Some Additional Notes on Mixture Toxicity of JP-5 Implicated Compounds

Additivity versus Nonlinear Interactions. It is generally assumed that acute general anesthesia, which is most likely associated with some sort of disruption of nerve cell membrane function through dissolution of narcotic compounds in these membranes (there are competing theories of general anesthesia, but to date none have been substantiated, and the one presented here can be regarded as the most parsimonious one), is an additive process, since no specific interactions appear involved and there is a very large receptor (cell membrane)/substrate ratio. Moreover, unless proof to the contrary is encountered, it is probably safe to assume that chronic Psycho-organic Solvent Syndrome is also an additive mechanism, expressed on an active-compound basis (i.e., excluding nontoxic metabolites).

Moreover, it has been and can be shown that even in mixtures of explicitly nonadditive (either synergistic, potentiating, or antagonistic) chemicals, the overall effect of a mixture tends toward an additive, narcotic limit for n approaching infinity, with n being the number of compounds in a mixture, provided the mixture has approximately equal distribution of compound mole fractions [(*72*), see also Berenbaum (*40*)]. This phenomenon is ascribed to the notion that for such a mixture individual compounds or groups of compounds acting on the same receptor or by the same mechanism, are generally present at concentrations below their pharmacologic/toxicologic threshold level. The one thing they then have in common is their ability to partition into membranes and elicit narcosis; since all compounds in principle have this ability, and simple narcosis is an additive phenomenon based on molar units, the associated joint toxic action is additive.

Conclusions

Over the last decades, a number of techniques have been developed, and a number of advances have been made in the fields of chemical, toxicological, and computational sciences that when combined offer for the first time in the study of the toxicology of chemical mixtures the possibility of actually predicting some aspects of the toxicity of said mixtures. In this paper we have introduced and briefly described three techniques we think will form the cornerstones of predictive toxicology for (complex) chemical mixtures in the near future. These three techniques are: QSAR analysis, which can be used to predict needed physicochemical and toxicological parameters for unknown compounds or for surrogate compounds; lumping analysis, which can drastically reduce the complexity of the description of a mixture while reporting the error introduced by a particular level of simplification so the artificially introduced error can be kept within reasonable limits; and PBPK/PD modeling, which can be used to describe the toxicokinetics (and possibly the toxicodynamics) of an ensemble of compounds or lumped pseudocompounds, including possible interaction effects. In combination, these three tools can be used to predict target organ/site levels of individual compounds or the entire mixture from exposure data—or conversely, they can be used to set safe exposure levels given threshold target site doses or dose–time combinations. This enables one to deal with the predicted toxicity of complex mixtures in a more integrated way than just focusing on one or two major components; moreover, it offers an approach to predict at least some toxicological aspects of unknown, or partially known, or variable mixtures.

We have illustrated this approach by describing how it can be applied to the integrated risk assessment of a navy jet fuel, JP-5, a complex mixture of mainly hydrocarbons. We realize that some parts of this example have been worked out more completely than others, and that we did not (yet) present an actual model for the toxicokinetics and dynamics of JP-5. We do, however, have a preliminary working model for the pharmacokinetics of hydrocarbon mixtures based on the hydrocarbon block method and the partition coefficient QSARs developed by De Jongh et al. (*35*), written in Advanced Continuous Simulation Language (ACSL) (*73*). A copy of this model is available from the authors on request.

Appendix: How to Minimize Error in Lumping

Lumping two or more discrete compounds into one pseudocompound to decrease the complexity of description of a mixture will introduce error into a model because the different physicochemical or other parameters for the separate compounds are being replaced by averaged parameters for the pseudocompound. How can this error be minimized? We discuss a method to do just that, employing a lumping approach for simple mono-exponential kinetic rate constants as an example; note that the same approach can be used for lumping on any parameter.

Single-component Representation of a Composite Linear System's Concentration: Discounted Least Squares

Let there be a linear system of the following form:

$$\frac{dC_j(t)}{dt} = -k_j C_j(t) \qquad \text{(A.1)}$$

so that

$$C_j(t) = C_j(0)e^{-k_j t}. \qquad \text{(A.2)}$$

Therefore, the total concentration at time t of all components is

$$\hat{C}(t) = \sum_{j=1}^{J} C_j(0)e^{-k_j t} = \int_0^\infty e^{-kt}\, dG(k) \qquad \text{(A.3)}$$

The latter representation is arrived at if we order the (positive) k_js in order of size, $k_1 < k_2 < \ldots\ k_J$, and think of $G(k)$ as having jumps (saltuses) of magnitude $C_j(0)$ at $k = k_1$, $k = k_2$, ..., $k = k_J$. This means that

$$\int_0^\infty dG(k) = G(\infty) = \sum_{j=1}^{J} C_j(0) = C$$

define $\bar{G}(k)$ as $G(k)/C$ so $\bar{G}(\infty)=1$. It can be seen that $\hat{C}(t)$ is the Laplace-Stieltjes transform of the mixing discrete distribution. This distribution summarizes the between-component variability in the current simple system.

Now consider replacing the above system with a single-component (S-C) system:

$$\hat{C}(t;\kappa) = Ce^{-\kappa t} \qquad (A.4)$$

The problem is to determine the (single) summarizing rate parameter, κ. We do so by assigning a penalty for lack-of-fit of the S-C representation to the true function.

Discounted Least-Squares Fit of Single Component

Put

$$S_1(\kappa;\theta) = \int_0^\infty \left(\hat{C}(t;\kappa) - \hat{C}(t)\right)^2 e^{-\theta t} dt. \qquad (A.5)$$

Notice that, for any κ, this considers an error or fit-discrepancy at every t, namely $\hat{C}(t;\kappa)-\hat{C}(t)$ and squares it; then each such squared error is multiplied by a discount factor, $e^{-\theta t}$ that, for $\theta > 0$, weights the early discrepancies more extensively than those occurring later in time. Finally, all such discounted squared discrepancies are added (integrated) to obtain the fit score, $S_1(\kappa;\theta)$; this value is the best single component to represent the mixture. Observe that

$$S_1(\kappa;\theta) = \int_0^\infty \left(Ce^{-\kappa t} - \int_0^\infty e^{-kt} dG(k)\right)^2 \cdot e^{-\theta t} dt. \qquad (A.6)$$

To minimize, differentiate on κ and equate the derivative to zero:

$$\frac{dS_1}{d\kappa} = \int_0^\infty 2\left(Ce^{-\kappa t} - \int_0^\infty e^{-kt} dG(k)\right) \cdot e^{-\theta t}(-)te^{-\kappa t} dt = 0. \qquad (A.7)$$

This leads to the equation

$$C\int_0^\infty te^{-(2\kappa+\theta)t} dt = \int_0^\infty dG(k) \int_0^\infty te^{-(2\kappa+\theta+k)t} dt. \qquad (A.8)$$

Perform the time-integration to find

$$\frac{C}{(2\kappa+\theta)^2} = \int_0^\infty dG(k) \frac{1}{(\kappa+\theta+k)^2} \qquad (A.9)$$

or

$$\int_0^\infty \frac{dG(k)}{C} \cdot \frac{(2\kappa+\theta)^2}{(\kappa+\theta+k)^2} \equiv \int_0^\infty d\bar{G}(k)\left(\frac{2\kappa+\theta}{\kappa+\theta+k}\right)^2 = 1, \qquad (A.10)$$

where we have used the fact that

$$\int_0^\infty dG(k) = C, \text{ so } \int_0^\infty d\bar{G}(k) = \bar{G}(\infty) = 1.$$

Notice that the factor

$$\left(\frac{2\kappa+\theta}{\kappa+\theta+k}\right)^2$$

increases from

$$\left(\frac{\theta}{\theta+k}\right)^2 < 1$$

at κ=0 to 4 when $\kappa\to\infty$; consequently, at least one solution of (A.10) always exists if $\bar{G}(k)$ has strictly positive support. Note that if $\bar{G}(k)$ concentrates at $k = k_1$, e.g., 3.2, and therefore there is just that single component, then (A.10) becomes

$$\left(\frac{2\kappa+\theta}{\kappa+\theta+k_1}\right)^2 = 1, \qquad (A.11)$$

which is only satisfied when $\kappa = k_1$. So our method is consistent to the degree that it recaptures a single component.

Next check for a two-component system. Put $C_1(0)=C_2(0)$, so

$$\int_0^\infty d\bar{G}(k)\left(\frac{2\kappa+\theta}{\kappa+\theta+k}\right)^2 = \frac{1}{2}\left(\frac{2\kappa+\theta}{\kappa+\theta+k_1}\right)^2 + \frac{1}{2}\left(\frac{2\kappa+\theta}{\kappa+\theta+k_2}\right)^2 = 1$$

$$\frac{1}{(\kappa+\theta+k_1)^2} + \frac{1}{(\kappa+\theta+k_2)^2} = \frac{1}{\frac{1}{2}(2\kappa+\theta)^2} \qquad (A.12)$$

or

$$L(\kappa) = R(\kappa). \qquad (A.13)$$

Both $L(\kappa)$ and $R(\kappa)$ are monotone decreasing, but

$$L(0) = \frac{1}{(\theta+k_1)^2} + \frac{1}{(\theta+k_2)^2} < \frac{2}{\theta^2} = R(0);$$

however, $L(\kappa)$ decreases less rapidly than $R(\kappa)$, so a single intersection occurs at $\hat{\kappa} > 0$. This is the desired single-component rate. It is, of course, a function of θ, and the larger is θ the less do the particular values k_1 and k_2 influence the result, since the more decisively large time effects are made insignificant. On the other hand, let $k_1 < k_2$, and let k_2 become very large. In the extreme case when $k_2 = \infty$, we must solve

$$\frac{1}{(\kappa+\theta+k_1)^2} = \frac{2}{(2\kappa+\theta)^2},$$

so

$$2\kappa+\theta = \sqrt{2}(\kappa+\theta+k_1);$$
$$(2-\sqrt{2})\kappa = (\sqrt{2}-1)\theta + \sqrt{2}k_1$$

Consequently,

$$\hat{\kappa} = \frac{(\sqrt{2}-1)\theta + \sqrt{2}k_1}{2-\sqrt{2}} \qquad (A.14)$$

and if $\theta = 0$ (smallest discounting),

$$\hat{\kappa} = \frac{\sqrt{2}k_1}{2-\sqrt{2}} = \frac{1.414k_1}{2-1.414} = 2.41k_1. \qquad (A.15)$$

The algorithm struggles to imitate the combined effect of a very fast component and a slower one by increasing the lower one's rate, and this is at least qualitatively appropriate.

Weighted Discounted Least-Squares Fit of Single Component

Review the above to see that there is freedom to alter the basic fit-score function, $S_1(\kappa;\theta)$ without destroying its tractability. We give some examples.

Example 1: Power-of-time (Gamma) Weighting/Discounting. Replace $e^{-\theta t}$ in (A.5) by

$$w(t;\theta;\beta) = e^{-\theta t}\frac{(\theta t)^{\beta-1}\theta}{\Gamma(\beta)}, \quad 0<\beta. \tag{A.16}$$

This essentially concentrates on errors near the time-value β/θ so it tends to find κ that centers $e^{-\kappa t}$ near the middle of the individual $e^{-k_j t}$ values, where $t \approx \beta/\theta$.

The analysis is tractable: differentiation on κ now gives

$$\frac{dS_1}{d\kappa} = \int_0^\infty 2\left(Ce^{-\kappa t} - \int_0^\infty e^{-kt}dG(k)\right) \cdot C(-)te^{-\kappa t}e^{-\theta t}\frac{(\theta t)^{\beta-1}}{\Gamma(\beta)}dt. \tag{A.17}$$

Set the derivative equal to zero to produce the equation

$$\int_0^\infty e^{-2\kappa t}\cdot e^{-\theta t}\frac{(\theta t)^\beta \theta dt}{\Gamma(\beta+1)} = \int_0^\infty d\bar{G}(k)\int_0^\infty e^{-(k+\kappa)t}\cdot e^{-\theta t}\frac{(\theta t)^\beta\theta dt}{\Gamma(\beta+1)}. \tag{A.18}$$

Known Laplace transform formulas now give

$$\left(\frac{\theta}{2\kappa+\theta}\right)^{\beta+1} = \int_0^\infty d\bar{G}(k)\left(\frac{\theta}{\kappa+\theta+k}\right)^{\beta+1}$$

or

$$\int_0^\infty d\bar{G}(k)\left(\frac{2\kappa+\theta}{\kappa+\theta+k}\right)^{\beta+1} = 1, \tag{A.19}$$

which can be solved for κ.

Example 2: Reverse-Exponential Discounting. Suppose we want a weight scheme that concentrates on and attempts to minimize all errors that occur at large times. One way is to replace $e^{-\theta t}$ in (A.5) with

$$w(t;\theta)=1-e^{-\theta t}. \tag{A.20}$$

This can be put into equations (A.6) to (A.9) and the resulting equation solved. Details are omitted.

Example 3: Fractional (Percent) Error Minimization. If

$$e^{-\kappa t} - \int_0^\infty e^{-kt}d\bar{G}(k)$$

is the absolute error made in estimating by $e^{-\kappa t}$, a version of fractional error in doing so is

$$\frac{e^{-\kappa t} - \int_0^\infty e^{-kt}d\bar{G}(k)}{e^{-\kappa t}} = 1 - e^{-\kappa t}\int_0^\infty e^{-kt}d\bar{G}(k). \tag{A.21}$$

Thus,

$$S_1(\kappa;\theta) = \int_0^\infty\left(1-e^{\kappa t}\int_0^\infty e^{-kt}d\bar{G}(k)\right)^2 w(t;\theta). \tag{A.22}$$

Differentiate; set result equal to zero:

$$\frac{\partial S_1}{\partial\kappa} = \int_0^\infty 2\left(1-e^{\kappa t}\int_0^\infty e^{-kt}d\bar{G}(k)\right)e^{-\theta t}te^{\kappa t} = 0. \tag{A.23}$$

We get

$$\frac{1}{(\theta-\kappa)^2} = \int_0^\infty d\bar{G}(k)\frac{1}{(\theta+k-2\kappa)^2}. \tag{A.24}$$

Solve for κ. Other weightings are also possible.

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